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STUDIES ON THE EFFECT OF FOREST INSECT CONTROL WITH ETHYLENE DIBROMIDE ON WATER QUALITY

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U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
Bureau of State Services
Division of Water Supply and Pollution Control
Region VIII

Colorado River Basin Water Quality Control Project

June 1963

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STUDIES ON THE EFFECT OF FOREST INSECT CONTROL WITH ETHYLENE DIBROMIDE ON WATER QUALITY

INTRODUCTION

At the request of the U. S. Forest Service Regional Office, Ogden, Utah, limited water quality investigations were made on the upper Green River by the Colorado River Basin Project in relation to possible effects from the Forest Service Moose Creek Insect Control operation conducted in the summer-fall of 1962. As this was a relatively new type of operation, it was believed that significant information would be obtained which would be useful for evaluating future operations of this kind within the Colorado River Basin.

During the period July 15 to October 15, 1962, approximately 5,500 acres of evergreen forests within the Green River watershed were treated for insect control by the U. S. Forest Service. The spray material used was a formulation of ethylene dibromide in fuel oil. Water quality investigations were conducted in the Green River and its tributaries before and during the spray operations.

SUMMARY AND CONCLUSIONS

The U. S. Forest Service treated approximately 5,500 acres of evergreen forest for spruce bark beetle control during the period from July 15 to October 15, 1962.

The spray material used was an ethylene dibromide - fuel oil - triton mixture containing 34 percent by weight ethylene dibromide. The spray material was mixed at a central mixing plant and transported by truck to a central point near the spray area. Individual infested trees were sprayed with stirrup pumps which were transported to the site by packhorse or on foot. Approximately 63,000 trees were treated with about 26,800 gallons of EDB concentrate in a 1:9 mixture with diesel oil.

The area treated was a steep, relatively inaccessible area within the Bridger National Forest and immediately west of the Green River Lakes, Wyoming. Drainage from the area treated was into the Green River or its major tributaries, Moose Creek and Gypsum Creek.

It was believed that heavy rains may wash some of the spray material into streams and have an effect on aquatic life or other water quality characteristics. Personnel from the Colorado River Basin Project conducted a laboratory and field study to determine the possible effects.

As the toxicity of ethylene dibromide was unknown, laboratory bioassays were conducted to determine the toxicity to fish. A 96-hour TL_m (medium tolerance limit) value of 7 mg/l was established as the toxicity of ethylene dibromide to rainbow trout. It is believed that a concentration exceeding 1 or 2 mg/l may be detrimental to aquatic life.

Field studies were conducted to determine the effect of the summer spray operation on stream bottom animals. Samples were taken at various stations before and after the operations. Bottom animal samples indicated no effect on aquatic life in the Green River or its tributaries. Likewise, water quality characteristics such as dissolved oxygen, pH, alkalinity and hardness were not affected.

A method of analysis for ethylene dibromide in water was developed using a gas chromatograph. After extraction with carbon tetrachloride, definite peaks on the chromatogram could be used to detect and estimate the concentration of ethylene dibromide in water samples to a minimum of 0.1 mg/l.

Five sets of water samples were taken at stream stations before spraying started, after heavy rainfall in the area, and after a substantial portion of the area had been sprayed. There was no apprent runoff of ethylene dibromide from sprayed areas. No detectable amounts were found in any water samples taken after the spray operations started and following heavy rainfall in the area. The only samples showing detectable quantities of ethylene dibromide were taken before spraying began and were located in drainage below the spray mixing plant. This may indicate some spillage or seepage from this site.

GENERAL DESCRIPTION OF AREA

The area treated for insect control is within the Bridger National Forest in west central Wyoming and is part of the Wind River mountain range. It consists of an evergreen forest on steep slopes mostly at elevations of between eight and ten thousand feet. The 5,500 acres treated during 1962 lie roughly between Sheep and Little Sheep Mountains and west of the Green River Lakes (Figure 1). Most of the area is rugged terrain and not readily accessible except by horseback or on foot.

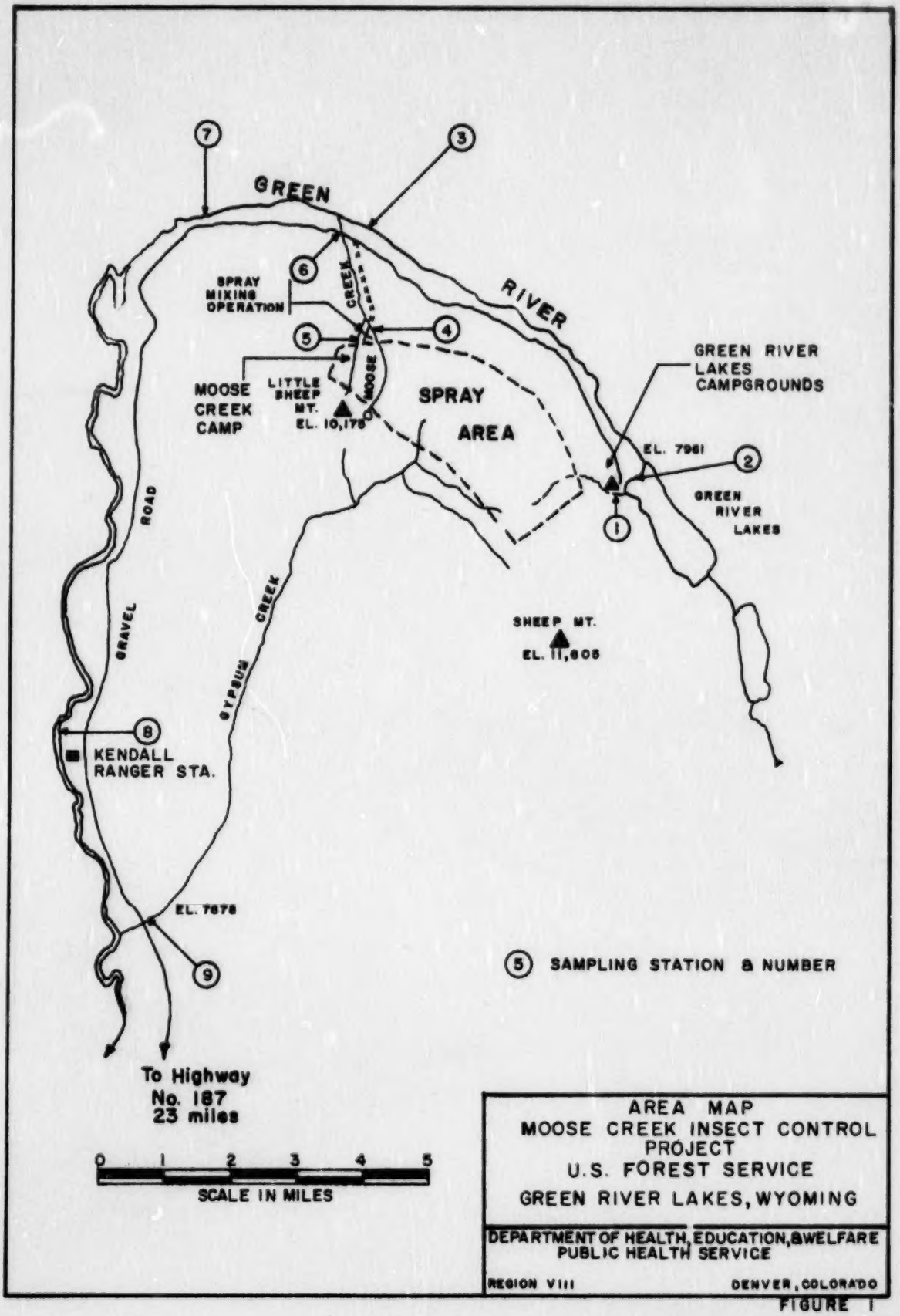
The area lies wholly within the Green River watershed with drainage directly into the Green River and its major tributaries, Gypsum Creek and Moose Creek.

FOREST SERVICE OPERATIONS

Individual trees within the spray area which were infested with spruce bark beetles were sprayed with ethylene dibromide formulation by means of stirrup pumps. The formulation was mixed in a central mixing plant (Figure 1) and transported by truck to a central area. From there, spray cans were transported by packhorse to the previously marked tree to be sprayed.

During the operation, from mid-July to mid-October, approximately 63,000 trees were sprayed with approximately 26,800 gallons of EDB concentrate. The concentrate consisted of approximately 17 gallons of EDB, 6 gallons of triton emulsifier, and 77 gallons of fuel oil per 100 gallons or approximately 34 percent by weight ethylene dibromide. The spray material as applied consisted of 1 part concentrate to 9 parts of diesel oil.

A base camp was maintained by the Forest Service at Moose Creek to house and feed the approximately 200 men participating in the spray operations.



AREA MAP
MOOSE CREEK INSECT CONTROL PROJECT
U.S. FOREST SERVICE
GREEN RIVER LAKES, WYOMING

DEPARTMENT OF HEALTH, EDUCATION, & WELFARE
PUBLIC HEALTH SERVICE

REGION VIII DENVER, COLORADO

FIGURE 1

COLORADO RIVER PROJECT STUDIES

As ethylene dibromide is slightly soluble in water, it was believed that heavy rains may wash some of the material into streams and possibly have an effect on aquatic life and other water uses. Major Project studies thus included biological investigations to determine possible effects on aquatic life and studies to detect and measure concentrations of EDB in stream waters.

BIOLOGICAL INVESTIGATIONS

Biological studies included laboratory bioassays to determine the toxicity of EDB to fish and field studies to determine the effect of the spray operation on aquatic life and pertinent water quality characteristics.

BIOASSAYS

No information could be found in the literature on the toxicity of ethylene dibromide to fish. It was therefore decided to conduct laboratory bioassays on samples of EDB to develop information on its toxicity to fish. The Forest Service furnished samples of the materials expected to be used in the spray operations. Two samples were furnished, one was 98 percent pure EDB and the other an EDB formulation containing 34 percent EDB in fuel oil with a triton emulsifier.

Bioassays were conducted by the routine procedure given in the eleventh edition of Standard Methods 1/. The test fish were 4-inch rainbow trout fingerlings and the diluent was Salt Lake City tap water. Tests were performed in a constant temperature room at 60°F.

Bioassays were conducted on the EDB formulation added directly to water and on the pure EDB in both water and acetone solutions. Because of the relatively low solubility of EDB in water (0.43 grams/100 grams at 30°C.), it was believed that a more even distribution in the test aquaria might be obtained with an acetone solution. However, the results in water and acetone were comparable.

Stock suspensions or solutions (0.1 to 1 percent by weight) of the materials were prepared and necessary quantities added to the test jars to prepare a logarithmic series of test concentrations (e.g., 32, 18, 10, 5.6, and 3.2 mg/1). Five fish were added to 20 liters of each concentration and fish reactions observed for a 96-hour period. From fish mortality in the different concentrations, TLm (median tolerance limit) values were estimated.

Bioassay results are shown in Table I. The results of chemical control tests and the data from which bioassay results were computed are shown in Appendix A.

From the results, it appears that the triton emulsifier and fuel oil contribute to some extent to the toxicity of the EDB formulation. On the basis of 34 percent EDB, the TLm value would be about 5 mg/1 while the actual value

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1/ Standard Methods for the Examination of Water and Wastewater, Eleventh Edition, American Public Health Association, New York (1960).

of EDB in water solution was about 7 mg/l. While EDB is fairly toxic to fish, it is not nearly as toxic as some other pesticides (e.g., DDT - TLm value - 0.016 mg/l) used for forest insect control.

The above values are concentrations that produce 50 percent mortality of a fairly sensitive fish in a short time period. Other organisms may be more sensitive and long-time exposure may increase the effect.

On the basis of past knowledge and experience, it may be expected that concentrations of EDB exceeding 1 or 2 mg/l may have a detrimental effect on fish or other aquatic life. Apparently the formulation has little effect on the other water quality characteristics measured.

FIELD STUDIES

Sampling stations were established as shown in Figure 1 and described in Table II. Water and bottom organism samples were collected at each station in July before spray operations started and again in September after about two thirds of the operation was completed.

Analyses for water quality were conducted on site with a chemical field kit and by conventional methods. Results showing water quality conditions before and after spray operations are shown in Table III. As may be expected, the spray operation had little or no effect on water quality characteristics. Slight increases in alkalinity may be explained by decreased stream flows. Dissolved oxygen values were at or near saturation at all stations during both sampling periods. Turbidity readings were not taken as all waters were very clear (USGS scale - less than 7) during both periods.

Stream flows in the Green River were much higher in July than in September. While there were no USGS gauges in the study area, values obtained from the USGS for flows near Daniel, Wyoming (approximately 25 miles below the study area), showed 1,530 cfs. in July and 214 cfs. in September. Flows in Gypsum Creek were also higher with an estimated flow of 20 cfs. in July and 8 cfs. in September. Flows in Moose Creek were not greatly different in July and September with an estimated flow of 3 cfs. about evenly divided between the two tributaries.

One or two square-foot bottom animal samples were collected with a Surber sampler from shallow, rocky, riffle areas at each station. Organisms were preserved with formalin and returned to the laboratory for sorting and identification.

Results of bottom animal samples (Table IV) indicate no major effect on bottom animals from the spray operations. While there were some differences in numbers and types, clean water organisms such as mayfly, stonefly, and caddisfly larvae were found at all stations. A greater abundance of organisms was found at Green River stations in September than in July. This probably reflects the concentration of larger numbers of organisms in a smaller area of river bottom as the stream flow decreased. Organisms were less abundant in Moose Creek than in the Green River. Whether this stream is just normally less fertile or reflects some past conditions (drouth, etc.) is not known. However, it appears that the summer pesticide operation had little effect on the aquatic fauna.

TABLE I

Results of Bioassays with EDB

Test Material	TLm - Median Tolerance Limit (mg/l) 24 hour	48 hour	96 hour
34% EDB formulation	14.5	14.5	14.5
98% EDB (water solution)	14.5	9.1	7.0
98% EDB (acetone solution)	13.5	9.1	8.1

TABLE II

Location of Sampling Stations

Station	
1	Small intermittent stream on the south side of Green River Lakes campground
2	Lower Green River Lake near outlet
3	Green River, one mile above entrance of Moose Creek
4	East Fork of Moose Creek above spray mixing plant
5	West Fork of Moose Creek above spray mixing plant
6	Moose Creek near mouth
7	Green River, two miles below mouth of Moose Creek
8	Green River at Whiskey Grove Camp, three miles above entrance of Gypsum Creek
9	Gypsum Creek near mouth

TABLE III

Analytical Results

Sta.	Date	Time	Temp. (°C.)	D.O. (mg/l)	pH	Alk. as $CaCO_3$ (mg/l)	Hard. as $CaCO_3$ (mg/l)	Cond. (μ mhos)
Before spray operation - July 16-17, 1962								
1	7/16/62	8:30A	4.5	8.8	7.7	4	80	120
2	7/16	9:30A	10	9.0	7.3	20	24	70
3	7/16	10:30A	11	9.2	7.7	16	20	62
4	7/16	2:30P	16	7.0	8.2	164	160	400
5	7/16	3:00P	12	7.6	8.2	164	156	360
6	7/16	11:00A	9.5	8.4	8.4	164	170	380
7	7/17	8:30A	7	8.6	8.4	24	18	55
8	No sample taken							
9	7/17	10:30A	5	9.6	8.4	100	110	220
After spray operation - September 14, 1962								
1	No sample taken - stream dry							
2	9/14/62	11:00A	13	9.6	7.5	22	28	-
3	9/14	1:30P	11	9.0	8.1	26	48	-
4	9/14	12:00M	8	9.2	8.1	208	200	-
5	9/14	12:30P	8	9.0	8.2	208	176	-
6	9/14	2:00P	8	9.0	8.3	200	205	-
7	9/14	3:00P	13	8.0	8.2	26	52	-
8	9/14	9:30A	11	9.6	8.0	66	68	-
9	9/14	3:30P	10	9.2	8.3	142	170	-

TABLE IV

Bottom Animals in One Square Foot Riffle Samples

Kind of Animal	Number Animals per Square Foot							
	Sta 1	Sta 3	Sta 4	Sta 5	Sta 6	Sta 7	Sta 8	Sta 9
Before spray operation July 16-17, 1962								
Ephemeroptera (Mayflies)	-	69	18	30	1	66	1/	121
Plecoptera (Stoneflies)	-	1	-	1	-	8	1/	1
Trichoptera (Caddisflies)	-	2	1	9	7	14	1/	33
Lepidoptera (Aquatic moths)	-	-	1	-	-	1	1/	-
Coleoptera (Aquatic beetles)	-	-	-	9	5	32	1/	25
Diptera (True flies)	241	52	142	11	8	-	1/	290
Oligochaeta (Aquatic worms)	-	-	3	3	-	-	1/	-
Total number	241	124	165	63	21	121		470
Weight in grams	0.24	0.88	0.65	0.74	0.14	1.20		2.13

TABLE IV (CONTINUED)

Bottom Animals in One Square Foot Riffle Samples

Kind of Animal	Number Animals per Square Foot						
	Sta 1	Sta 3	Sta 4	Sta 5	Sta 6	Sta 7	Sta 8	Sta 9
After spray operation September 14, 1962								
Ephemeroptera (Mayflies)	2/	179	11	53	17	106	72	245
Plecoptera (Stoneflies)	2/	16	50	114	3	24	19	9
Trichoptera (Caddisflies)	2/	96	5	13	4	17	55	37
Lepidoptera (Aquatic moths)	2/	-	-	-	-	-	-	-
Coleoptera (Aquatic beetles)	2/	7	-	38	28	2	39	15
Diptera (True flies)	2/	68	22	7	30	7	4	35
Oligochaeta (Aquatic worms)	2/	2	1	-	-	-	2	2
Gastropoda (Snails)	2/	-	-	-	-	-	6	-
Total number		368	89	225	82	156	197	343
Weight in grams		3.16	0.13	0.31	0.11	2.26	2.63	1.23

1/ No sample taken
2/ No sample taken - stream dry

ANALYSIS FOR EDB IN WATER

In order to analyze water for EDB concentrations, it was necessary to conduct laboratory investigations to develop a suitable method. Following this, stream water samples were collected in the field and returned to the laboratory for analyses.

Laboratory Investigations

Known samples of EDB were subjected to gas chromatography and a definite peak established. The method is described in detail in Appendix B.

Ethylene dibromide is a commercial material which consists of a number of organic species, as evidenced by gas chromatography of the commercial sample. The largest peak has been assumed as the ethylene dibromide fraction. Since the method utilizes carbon tetrachloride as a solvent for extracting the ethylene dibromide from the water sample (see Appendix B), the sample to be injected consists of a small concentration of ethylene dibromide in carbon tetrachloride. Figure 2 shows the chromatogram which includes the major peak (a) due to carbon tetrachloride followed by peak (b) on the tail due to the ethylene dibromide. Peak height correlates rather well with the quantity of ethylene dibromide.

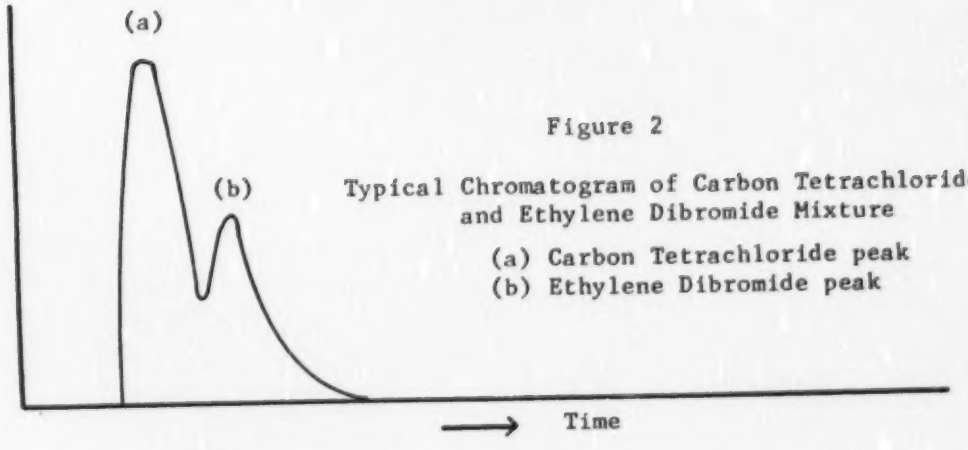

Figure 2

Typical Chromatogram of Carbon Tetrachloride and Ethylene Dibromide Mixture

(a) Carbon Tetrachloride peak
(b) Ethylene Dibromide peak

Small peaks at (b) must be considered the lower limit of the results. This lower limit is estimated at approximately 0.1 mg/l as ethylene dibromide. Thus, the results obtained would indicate values ranging from a low of 0.1 mg/l upward.

Field Samples

Five sets of water samples were taken at sampling stations shown in Figure 1 and described in Table II. A set of samples was collected on each of the following dates:

First Sampling	-	July 15
Earliest Spraying	-	July 23
Second Sampling	-	August 15
Third Sampling	-	August 18
Fourth Sampling	-	August 19
Fifth Sampling	-	August 22

The sampling time for the most probable detection of ethylene dibromide in streams of the area was estimated at one and two days after a heavy rainfall and subsequent to the spraying of about 2,000 acres of forest. The second sampling was made on August 15 after some 2,000 acres had been treated. A heavy rainstorm took place on August 17 and Forest Service personnel obtained the third and fourth sets of samples on each of the two following days at five stations. A fifth set of samples was obtained on August 22 at six stations.

The results of analyses of the samples are shown in Table V.

Two samples showed measurable concentrations of ethylene dibromide, station 6, first sampling, and station 7, first sampling. All other samples collected showed values reported at less than 0.1 mg/l.

No detectable amounts of ethylene dibromide were found in any water samples taken after the spraying was started. The only samples showing detectable quantities of ethylene dibromide were located in a drainage direction leading away from the mixing station on Moose Creek and Green River. These values show some evidence of spillage or seepage of ethylene dibromide from the mixing site.

TABLE V

Results of Analyses for EDB

Station	Sampling 1st	2nd	3rd	4th	5th
1	n.d.	n.d.	-	-	n.d.
2	n.d.	n.d.	-	-	n.d.
3	n.d.	n.d	n.d.	n.d.	-
4	n.d.	n.d.	n.d.	n.d.	n.d.
5	n.d.	n.d.	n.d.	n.d.	n.d.
6	< 1.3 mg/l	n.d.	n.d.	n.d.	n.d.
7	< 2.1 mg/l	n.d.	n.d.	n.d.	n.d.
8	-	-	-	-	n.d.

n.d. - not detected

APPENDIX A

BIOASSAY DATA

TEST MATERIAL - EDB formulation - EDB in fuel oil with triton emulsifier (Approx. 34% EDB) Sp.Gr. - 1.05 - Stock suspension in water (0.1%) 1 ml/1.

DATE - 5/28-31/62

TEST FISH - Rainbow trout fingerlings (4") 5 in 20 liters

Temp. - 60°F.

DILUTION WATER - Salt Lake City tap

TEST CONCENTRATION (mg/1)	ml. STOCK	TIME OF DEATH (Hrs.) 1st	TIME OF DEATH (Hrs.) ALL	% SURVIVAL 24 HRS.	48 HRS.	72 HRS.	96 HRS.
32	320	2	24	0	0	0	0
18	180	27	-	20	20	20	20
10	100	-	-	100	100	100	100
5.6	56	-	-	100	100	100	80
Control	-	-	-	100	100	100	100
TLm (Median Tolerance Limit)				14.5	14.5	14.5	14.5

CHEMICAL DATA

TEST CONCENTRATION (mg/1)	TIME	D.O. (mg/1)	pH	ALK. as $CaCO_3$ (mg/1)	ACID as $CaCO_3$ (mg/1)	HARD. AS $CaCO_3$ (mg/1)	SPEC. COND. (μ mhos)
32	Initial	7.8	8.3	98	4	150	290
	27 hrs.	3.8	7.3	108	8	150	270
18	Initial	7.8	8.3	102	4	145	290
	96 hrs.	2.2	7.5	102	4	135	275
10	Initial	7.8	8.3	96	4	155	290
	96 hrs.	2.6	7.6	110	12	150	280
5.6	Initial	7.8	8.3	-	4	145	290
	96 hrs.	1.6	7.5	106	14	150	280
Control	Initial	7.8	8.2	-	4	150	290
	96 hrs.	2.0	7.6	106	10	145	280

SYMPTOMS AND REMARKS: Dissolved oxygen low but fish survived

APPENDIX A (CONTINUED)

BIOASSAY DATA

TEST MATERIAL - Ethylene dibromide (98%) DATE - 5/28-31/62
Sp.Gr. - 2.172 in water solution
Stock solution - 0.1% by weight (0.46 ml/1.) Temp. - 60°F.

TEST FISH - Rainbow trout fingerlings (4")
5 in 20 liters

DILUTION WATER - Salt Lake City tap

TEST CONCENTRATION (mg/1)	ml. STOCK	TIME OF DEATH (Hrs.) 1st	ALL	% SURVIVAL 24 HRS.	48 HRS.	72 HRS.	96 HRS
32	320	-	27	0	0	0	0
18	180	27	44	20	0	0	0
10	100	44	96	100	40	20	0
5.6	56	96		100	100	100	80
Control	-	-	-	100	100	100	100
TL_m (Median Tolerance Limit)				14.5	9.1	8.1	7.0

CHEMICAL DATA

TEST CONCENTRATION (mg/1)	TIME	D.O. (mg/1)	pH	ALK. as $CaCO_3$ (mg/1)	ACID as $CaCO_3$ (mg/1)	HARD as $CaCO_3$ (mg/1)	SPEC. COND. (μ mhos)
32	Initial	7.2	8.2	-	4	155	295
	27 hrs.	4.6	7.8	114	4	150	285
18	Initial	7.2	8.2	-	4	155	300
	27 hrs.	3.0	7.7	120	4	150	285
10	Initial	7.2	8.2	-	4	140	300
	96 hrs.	3.4	7.7	118	12	145	290
5.6	Initial	7.2	8.2	-	4	155	300
	96 hrs.	2.0	7.6	122	12	155	300
Control	Initial	7.8	8.2	-	4	140	290
	96 hrs.	2.0	7.6	106	10	145	280

SYMPTOMS AND REMARKS: Dissolved oxygen low but fish survived

APPENDIX A (CONTINUED)

BIOASSAY DATA

TEST MATERIAL - Ethylene dibromide
1% acetone solution
Stock - 0.46 ml per 100 ml acetone

DATE - 5/28-31/62

Temp. - 60°F.

TEST FISH - Rainbow trout fingerlings (4")
5 in 20 liters

DILUTION WATER - Salt Lake City tap

TEST CONCENTRATION (mg/l.)	ml. STOCK	TIME OF DEATH (Hrs.) 1st	ALL	% SURVIVAL 24 HRS.	48 HRS.	72 HRS.	96 HRS.
32	32		27	0	0	0	0
18	18		27	0	0	0	0
10	10	44	-	100	40	20	20
5.6	5.6			100	100	100	100
Control	-			100	100	100	100
TLm (Median Tolerance Limit)				13.5	9.1	8.1	8.1

CHEMICAL DATA

TEST CONCENTRATION (mg/l.)	TIME	D.O. (mg/l.)	pH	ALK. as $CaCO_3$ (mg/l.)	ACID as $CaCO_3$ (mg/l.)	HARD as $CaCO_3$ (mg/l.)	SPEC. COND. (μ mhos)
32	Initial	7.2	8.3	114	4	180	300
	27 hrs.	5.4	7.8	120	2	160	285
18	Initial	7.2	8.3	106	4	160	300
	27 hrs.	5.0	7.8	120	4	145	285
10	Initial	7.2	8.3	110	4	140	300
	96 hrs.	4.8	7.7	124	8	165	290
5.6	Initial	7.2	8.3	108	4	140	280
	96 hrs.	2.8	7.6	118	10	150	290
Control	Initial	7.4	8.2	-	4	155	290
	96 hrs.	3.4	7.7	112	8	145	285

APPENDIX B

TENTATIVE PROCEDURE FOR THE DETERMINATION OF ETHYLENE DIBROMIDE IN WATER

1. General Discussion

1.1 Principle: This method depends upon the solvent extraction of ethylene dibromide by carbon tetrachloride from the water sample. The extract is then subjected to gas chromatography with flame ionization detection in which the ethylene dibromide peak appears on the shoulder of the carbon tetrachloride peak.

1.2 Minimum detectable quantity: 2×10^{-8}g.

2. Apparatus

2.1 Gas chromatograph with hydrogen flame ionization detector. F and M Model 609 or equivalent.
2.2 Gas chromatograph column, 1/8" ID x 2' stainless steel filled with 20% silicone gum rubber on chromosorb W properly prepared and conditioned.
2.3 Graduated cylinder, 2000 ml. with glass stopper.
2.4 Centrifuge taking tubes 35 mm x 100 mm.
2.5 Syringe, 50 µl. capacity
2.6 Smith weighing buret

3. Reagents

3.1 Carbon tetrachloride. Spectroquality reagent treated with 1% activated charcoal for at least 15 minutes. This should be purified as needed since removal of the inhibitor will permit decomposition of the solvent.
3.2 Ethylene dibromide. Should be representative of the material actually analyzed since the commercial product consists of several species.

4. Procedure

4.1 Preparation of standards for the calibration curve: Add ethylene dibromide to the Smith weighing buret and transfer approximately 40 mg. of the reagent to a 500 ml. volumetric flask containing 200 ml. of pure CCl_4. Record the actual weight of the sample transferred. Dilute to the mark with carbon tetrachloride and mix well. Prepare a 1-1 dilution of this solution (1/2) and a 1-1 dilution of the (1/2) to make (1/4) and a 1-1 dilution of the (1/4) to make (1/8). With these four solutions, 10 µl aliquots may be subjected to gas chromatography to determine the location and height of the peak. Plot peak heights versus weight of ethylene dibromide and draw a smooth curve through these points.
4.2 Extraction of samples: To a 2000 ml. graduated cylinder, add 1000 ml. of water sample. Add 25-50 g. of ice in order to keep evaporation of the carbon tetrachloride to a minimum. Add 10 ml. of purified carbon tetrachloride. Securely stopper the cylinder and rock the cylinder gently about once every second for 15 minutes. Care must be

taken to avoid the blowing out of the stopper by pressure of carbon tetrachloride. After the rocking is completed, let the solution stand for 15 minutes. Decant off most of the water and transfer the carbon tetrachloride and some of the water to a 1" x 4" screw cap vial. Place the vial in the centrifuge tube, balancing the several other tubes as required. Centrifuge for 15 minutes at 1000-1500 rpm. These tubes will not break provided rubber cushions and water are used for support.

4.3 Chromatographing the samples. Adjust flow rates as follows: helium, 100 ml./min; hydrogen, 50 ml./min; and air, 200 ml/min. Raise the chromatograph column to 75°C. and allow 10-15 minutes to equilibrate. Run 10 µl pure CCl_4 blank to check out column performance. Inject 10 µl samples, each as soon as the pen has returned to the base line. Run a set of standards (section 4.1) for each set of samples since the use of a permanent calibration curve is not recommended.

5. Calculation

From the peak heights, determine the weight of ethylene dibromide in 10 ml. of each sample.

$$\text{mg/l. ethylene dibromide} = \frac{\text{Wt. in 10 ml.} \times \frac{10.00}{.01 \times 1000}}{1.0}$$

6. Accuracy

With a peak height of 5 mm or more, variation expected may run $\pm$ 10% or less. If the peak height is less than 5 mm, the variation expected could run as high as $\pm$ 50%.